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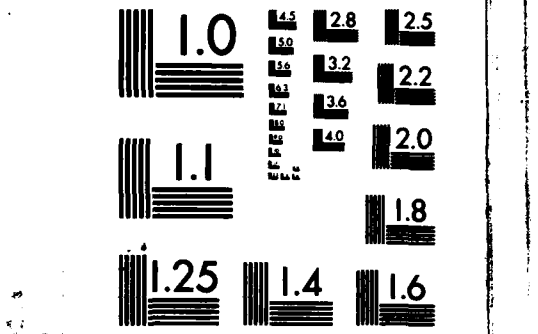

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"Comparative Studies on the Structure of Human Adenovirus Genomes 4, 7 and 21" (U).

Final Report

by

Radha Krishnan Padmanabhan, Ph.D.

December 1981

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701
Contract No. DAMD 17-79-C-9102

Prepared by

University of Maryland School of Medicine
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18. SUPPLEMENTARY NOTES The work supported by USAMRDC has resulted in 2 publications, 1 manuscript submitted for publication and 2 manuscript in preparation. Moreover, a portion of the work was reported at the annual meeting of the American Society for Biological Chemists, held at St. Louis (May 31-June 4, 1981) and at the West

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Physical mapping of Ad4 prototype (ATCCVR$_4$), Ad7 (Greider) and Ad7 (vaccine) strains with restriction endonucleases; DNA sequence analysis of the regions of Ad4 and Ad7 near their termini that seem to play a role in DNA replication; A new sensitive method to radioactively label the viral DNA in vitro; An improved method of plaque assay for titering the infectivity of enteric coated Ad4 and

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Ad7 (Greider) DNA was cleaved with eleven restriction endonucleases and their cleavage sites on the viral DNA were mapped using a sensitive method developed in our laboratory. Comparison of the cleavage patterns of Ad7 (Greider) with those of Ad7 (vaccine) DNAs reveal that the two are identical but exhibit some differences to the prototype strain of Ad7 (Gomen) DNA. Ad7 DNA from the prototype strain (ATCC, VR$_4$) was mapped for the cleavage sites of nine restriction endonucleases using similar methods.

The regions near the termini of all adenovirus genomes seem to play an im-

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18. (cont'd) Central States Biochemistry Conference, held at Lincoln, Nebraska (Oct. 23-24, 1981).

19. (cont'd) Ad7 vaccine tablets.

20. (cont'd)
important role in DNA replication. Comparison of our sequence data from these regions of Ad7 (Greider) and Ad4 (prototype) DNAs with our previous data on Ad2 and Ad12 DNAs reveals that there is a striking homologous seuqence of 14 base pairs present near the termini of all human Ad DNAs so far studied. This region may be important in the control of DNA replication in human cells.

We have optimized the conditions for titering the Ad vaccine strains present in enteric coated tablets. The use of human embryonic kidney cells as the host and the inclusion of dimethyl sulfoxide and DEAE-dextran in the agar overlay medium are recommended in the plaque assay.

TABLE OF CONTENTS

"Comparative Studies on the Structure of Human

Adenovirus Genomes 4, 7 and 21"

Final Progress Report

by

Radha Krishnan Padmanabhan, Ph.D.

(For the period July 1, 1979 - August 31, 1981)

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701
Contract No. DAMD 17-79-C-9102
University of Maryland School of Medicine
Department of Biological Chemistry
Baltimore, Maryland 21201

OBJECTIVES

The overall goal of the project is to study the genomic structure of Adenovirus prototype strains 4, 7 and 21 using several restriction endonucleases and compare their characteristic cleavage maps with those of the corresponding vaccine strains. These three serotypes of adenovirus have been shown to be involved in respiratory diseases among military recruits. Serotype 4 differs from 7 and 21 in that it does not produce tumor in animals, whereas 7 and 21 induce tumors at a moderate frequency. The purpose of the investigation is to develop a non-oncogenic recombinant strain in which the immunogenic regions of Ad7 (or Ad21) is preserved and the oncogenic region of Ad7 is substituted with the corresponding region from Ad4. The goal is feasible in view of the fact that the oncogenic and immunogenic regions have been shown to reside in different parts of adenovirus genome.

A second objective of the project is to develop a plaque assay for titering the infectivity of Ad4, 7 and 21 vaccine strains in the forms of enteric coated capsules.

INTRODUCTION

Human adenoviruses have been classified under DNA Tumor Virus Group since they produce tumor in newborn hamsters. However, there is no evidence so far to link them as causative agents in human cancers. All human adenoviruses, except probably Ad4 (since the data on Ad4 is lacking), can transform rat embryo cells in vitro and the transformed cells are tumorigenic when injected to newborn hamsters. All early experiments on in vitro transformation of adenoviruses were conducted with cells of non-human origin, such as rat embryo, and the difficulty of using human cells was due to the permissiveness of the cell for the replication of the virus. However, it was shown by Graham et al. (Ref. 1) that sheared Ad5 DNA could transform human embryonic kidney cells in vitro. The resulting transformed cells (293 cells) are now used for (1) isolating mutants of Ad5 defective in transformation due to the presence of integrated left-end of Ad5 DNA in 293 cells complementing the defective genes for transformation in the mutant (such mutants being unable to replicate in other human cells such as the HeLa or human KB cells); and (2) use as a host for superinfection by other adenovirus serotypes and titering their infectivities by plaque assay.

The conventional techniques for identification of adenovirus types are hemaagglunination inhibition and neutralization of infectivity. These techniques rely on reaction of antibodies with antigenic determinants on gene products which represent limited regions of the genome. Another approach for the identification of adenovirus types is based on the characteristic restriction endonuclease cleavage patterns exhibited by DNA from each serotype.

RATIONALE

The purpose of the project is to establish an alternate classification of sero-subtypes of adenoviruses based on restriction enzymes on the genomes of the vaccine strains of Ad4, 7 and 21. It is important to isolate transformation-defective vaccine strain of adenovirus because (1) it has been shown that wild type sheared Ad5 DNA can transform human cells in vitro; and (2) Ad7 and Ad21 form, during their infection, large proportion of incomplete virus particles containing DNA with the oncogenic region intact and hence potentially capable of transforming human cells during the gastrointestinal infection by the live enteric coated capsules.

PROGRESS REPORT

A. Summary of the two annual reports submitted earlier

1. Adenovirus Type 7 (Greider Strain) was grown in human KB cells. The virions were purified by CsCl equilibrium centrifugation, the DNA was extracted and purified. The viral genome was analyzed by restriction endonucleases EcoRI, BamHI, SmaI, HpaI, BclI, BstEII, and KpnI. The restriction endonuclease patterns of the viral DNA was compared with those of three Ad7 subgroups published by Wadell and Varsanyi. Patterns we obtained by cleaving Ad7 with SalI, HpaI, BamHI and SmaI were identical to those obtained with strain 1059 and strain 55142 (Ad7 vaccine strain) reported by Wadell and Varsanyi. However, EcoRI pattern of Ad7 (Greider) was different from strain 1059 and 55142 in that one site mapped at 85.4 units from the left end of the DNA is missing (Ref. 2, 3).

2. DNA sequence at the inverted terminal repetition of Ad7 DNA was analyzed in order to compare Ad7 (Greider) with Ad3 DNA, another member of "weakly oncogenic" group (B). Ad2 (and Ad5) member of non-oncogenic group (C) and with Ad12, member of "highly oncogenic" group (A) of human adenoviruses. Our results indicate that the length of the inverted terminal repetition of Ad7 (Greider) is identical to Ad3, but different from Ad2 (or Ad5) and Ad12 (or Ad18). The nucleotide sequence homology in this unique region between Ad7 and Ad3 is 95% (Ref. 4).

3. We purified the Ad7 DNA-protein complex to establish conditions for DNA-transfection in Human Embryonic Kidney cells in vitro. We labeled the protein moiety with ^{125}I on tyrosine residues. We discovered that tyrosine-labeled peptide was still found to be covalently attached to the DNA even after pronase and protease K treatments. This finding enabled us to develop a highly sensitive method to label DNA from human adenoviruses at their termini and physically map the viral genome with several restriction endonucleases. The use of this method in physical mapping is demonstrated by constructing the physical map of Ad7 (Greider) cleaved with BclI (Ref. 5).

4. A sensitive method was developed in our laboratory to label the 5' termini of Ad DNA which was found to be applicable to Ad7, Ad4 and Ad12 DNA due to the presence of a tyrosine-containing peptide covalently attached to these DNA molecules. We used this method to map the cleavage siges of Ad7 (Greider) DNA with ten restriction endonucleases (EcoRI, HindIII, BamHI, BclI, BstEII, XbaI, SmaI, HpaI, KpnI, XhoI) (Ref. 3).

5. Ad7 vaccine strain was passaged in 293 cells and then grown in large amounts in suspension cultures of KB cells. The DNA was extracted and purified. Restriction enzyme analysis of vaccine and Greider Ad7 strains revealed that the two strains gave identical cleavage patterns with 8 restriction enzymes.

6. Ad4 prototype strain (ATCC) have been passaged in 293 cells and then grown in large amounts in suspension cultures of human KB cells. The DNA from these virions have been extracted and purified. Using our sensitive method of terminal labeling, we have mapped the terminal restriction fragments of Ad4 (ATCC strain) with several restriction enzymes. We have also mapped the cleavage sites of 9 restriction endonucleases (see Section B) (See ref. 6).

7. Ad21 vaccine strain has been passaged in 293 cells and then the virus was grown in KB cell suspension cultures. We are currently in the process of isolating the DNA and analyzing its genomic organization with several restriction enzymes.

8. We analyzed the efficiencies of different protocols for titering the live Ad vaccine strains Ad4, Ad7 and Ad21 present in enteric coated tablets. We used HeLa cells, 293 (human embryonic kidney cells transformed by Ad5), KB cells and 549 cells as the host. We found that 293 cells are the most suitable for the plaque assay of these viruses. We compared different protocols for the overlay medium used in the titration procedure such as the high Mg^{2+} concentration (Williams, 1970), dimethyl sulfoxide alone, and dimethyl sulfoxide in the presence of 0.01% DEAE-dextran (McCown et al., 1979). From our studies, it is evident that a combination of 1% DMSO and 0.01% DEAE-dextran is the most effective in enhancing the plaque formation in 293 cells. We have recently obtained evidence that an overlay containing 1% DMSO and 0.01% DEAE-dextran enhances the transfection of Ad7 DNA into 293 cells (Ref. 9).

B. Progress report (Feb. 3, 1981 - Aub. 31, 1981)

1. Physical mapping of Ad4 prototype strain (ATCC) with nine restriction endonucleases (see Fig. 1)

At the time the annual progress report was prepared, we had just started the physical mapping of Ad4 DNA (prototype strain) and we had completed only the estimation of the sizes of the terminal fragments using the novel terminal labeling technique developed in our laboratory. Since then, we have completed the physical map of Ad4 DNA with 9 restriction endonucleases.

The methodology used to map the cleavage sites of the restriction enzyme on Ad4 DNA is the same as described for Ad7 (Greider) or Ad7 (vaccine) DNA described in detail in the second annual Progress report (page 5).

Sizes of the terminal fragments generated by each restriction enzyme

[^{125}I]labeled Ad4 DNA was digested with various restriction enzymes - BclI, HindIII, EcoRI, HpaI, XbaI, KpnI, XhoI, BamHI, and BstEII. The ethiduim bromide fluorescence of DNA fragments was photographed and [^{125}I] radioactivity was detected by autoradiography. From these data it was possible to calculate the sizes of the DNA fragments generated by each restriction endonuclease and in addition, to detect the labeled terminal fragments. Since BclI cleaves Ad4 DNA only once at 86.9 map units, by cleaving the labeled BclI-A and B fragments with each restriction enzyme, it was possible to deduce the orientation of the terminal fragments with reference to BclI-A or B terminus.

The physical map of all the cleavage sites of each restriction enzyme was deduced by mixed hydrolysis of Ad4 DNA with 2 restriction endonucleases, the cleavage sites of one of which had already been deduced unambiguously. The sizes of the DNA fragments generated in the mixed hydrolysis are given in Table 2. In some cases, the physical map deduced by mixed hydrolysis of Ad4 DNA with 2 restriction enzymes, was confirmed by the technique of partial hydrlysis of a single-end labeled DNA fragment with a restriction enzyme (Fig. 1).

2. DNA sequence analysis at the inverted terminal repetitions of Ad4 DNA

The rationale for analyzing the nucleotide sequence at the termini of Ad4 DNA comes from our previous studies on the comparative sequence analysis of inverted terminal repetitions of DNA from an adenovirus serotype from each of the three groups; A (highly oncogenic), B (weakly oncogenic), and C (non-oncogenic). There seemed to be a correlation between the length of inverter terminal repetition (ITR) of an Ad serotype and its oncogenicity in newborn hamsters.

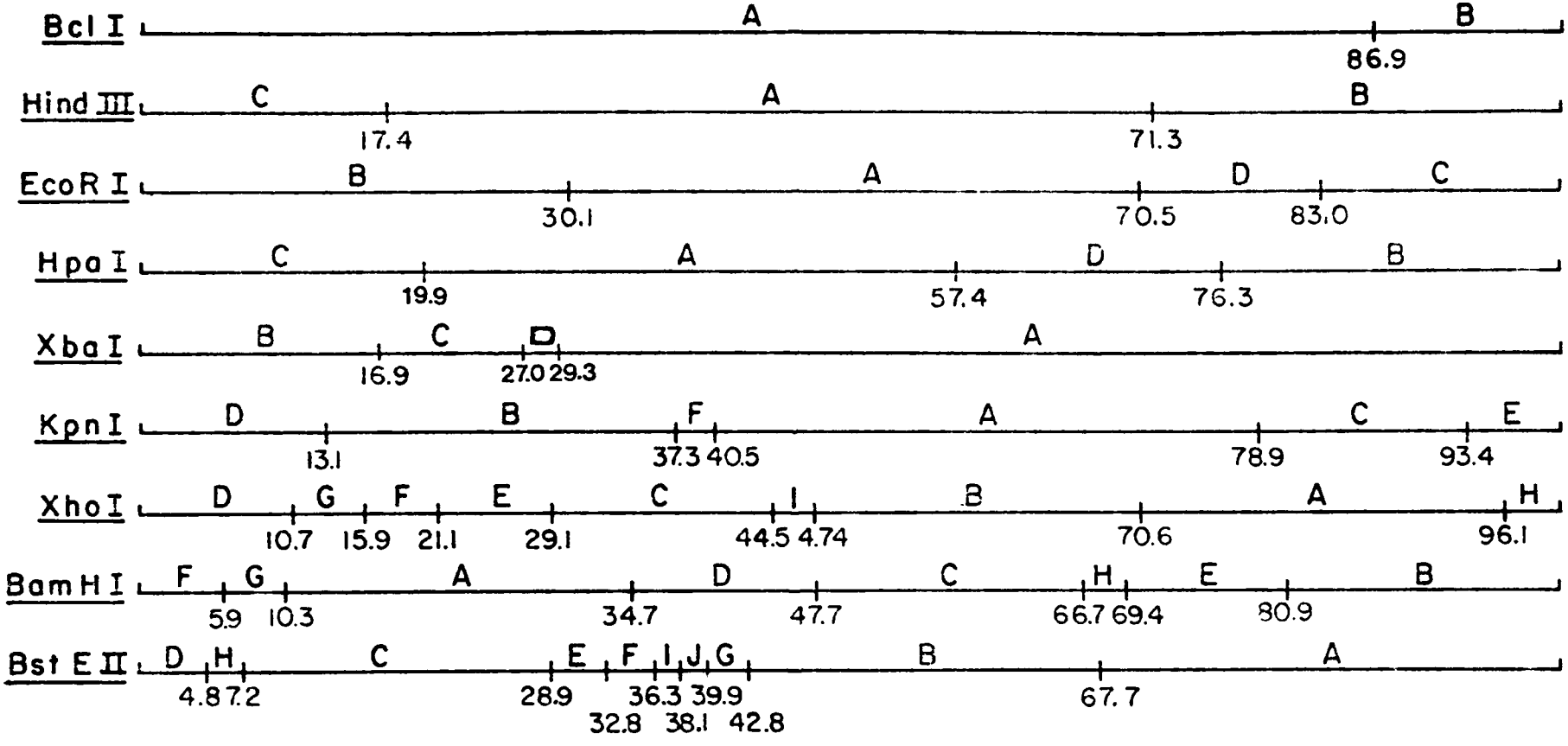
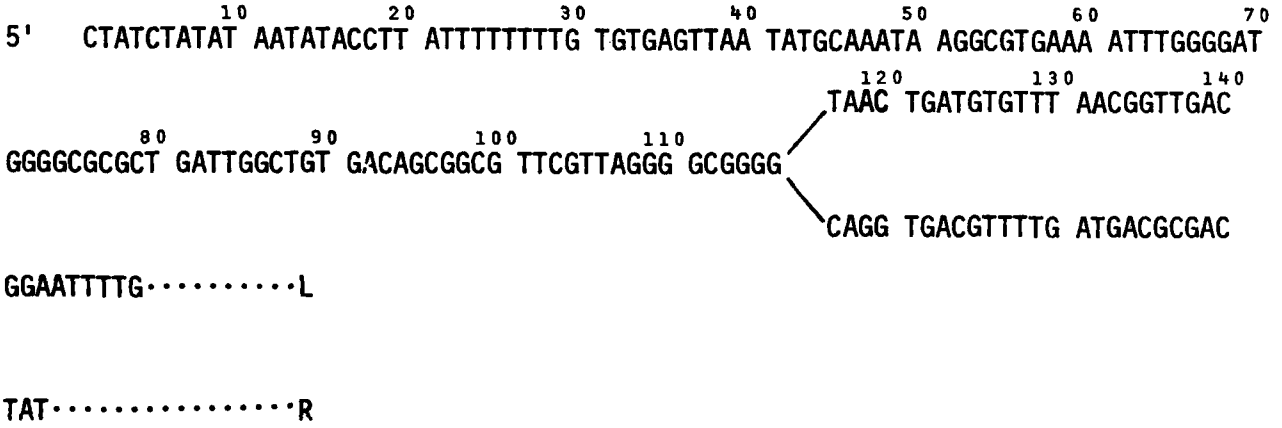

Bcl I
A
B
86.9
Hind III
C
A
B
17.4
71.3
EcoR I
B
A
D
C
30.1
70.5
83.0
Hpa I
C
A
D
B
19.9
57.4
76.3
Xba I
B
C
D
A
16.9
27.0 29.3
Kpn I
D
B
F
A
C
E
13.1
37.3 40.5
78.9
93.4
Xho I
D
G
F
E
C
I
B
A
H
10.7
15.9
21.1
29.1
44.5 4.74
70.6
96.1
BamH I
F
G
A
D
C
H
E
B
5.9
10.3
34.7
47.7
66.7 69.4
80.9
Bst E II
D
H
C
E
F
I
J
G
B
A
4.8 7.2
28.9
36.3 39.9
32.8
38.1
42.8
67.7

FIGURE 1

The ITR is shortest in non-oncogenic (Ad2 and Ad5) serotypes (103 base-pairs) and longest in highly oncogenic (Ad12 and Ad18) Ads. The length of ITR for the waekly oncogenic group B (Ad3 and Ad7) falls in the middle (136 base-pairs). Ad4 has been reported to be non-oncogenic but it is classified into a unique group E from the DNA-DNA homology data. The length of the ITR has been determined by us by sequence analysis to be 118 base-pairs (Fig. 2). Although the length of ITR of Ad4 DNA is smaller than those of weakly oncogenic Ad3 and Ad7, it is greater than group C Ads, 2 and 5. The significance of this finding is unknown at present.

FIGURE 2



Sequence at the inverted terminal repetition of Ad4

Table I. Restriction fragments of Ad4 (VR-4) strain

ag-nt	BclI	HindIII	EcoRI	HpaI	XbaI	KpnI	BamHI	XhoI	BstEII
A	86.9[l]	53.9	40.4	37.6	62.7[r]	38.4	24.4	25.5	32.3[r]
B	13.1[r]	28.7[r]	30.1[l]	23.6[r]	16.9[l]	24.6	19.1[r]	23.2	24.9
C		17.4[l]	17.0[r]	19.9[l]	10.1	14.5	19.0	15.4	21.7
D			12.5	18.9	2.3	13.1[l]	13.0	10.7[l]	4.8[l]
E						6.6[r]	11.5	8.0	3.9
F						2.8	5.9[l]	5.2	3.5
G							4.4	5.2	2.9
H							2.7	4.9[r]	2.4
I								2.9	1.8
J									1.8

l, r = left and right terminal fragments, respectively. These were identified by autoradiography of ^{125}I labeled DNA digest. Fragment lengths were estimated by electrophoretic mobility compared to fragments of known sizes. These numbers are expressed as "% of genome length."

Table II. Endonuclease R fragments produced by double digestion.

BclI + BamHI	BclI + BstEII	BclI + EcoRI	BclI + HindIII	BclI + HpaI	BclI + KpnI	BclI + XbaI	BclI + XhoII	HpaI + XhoI	HpaI + HindIII	HpaI + EcoRI	HpaI + XbaI	KpnI + HindIII	KpnI + EcoRI	KpnI + XbaI	BstEII + XbaI	BstEII + KpnI
24.4	24.9	40.4	53.9	37.6	38.4	59.5	23.2	18.3	37.6	27.6	30.1	30.8	30.0	38.4	32.9	24.6
19.0	21.7	30.1	17.4	19.9	24.2	16.9	16.3	15.3	23.6	19.9	23.6	19.9	17.0	14.5	24.6	15.6
13.0	19.2	13.1	15.6	18.9	13.1	13.1	15.4	12.9	17.4	17.0	18.9	14.5	13.1	13.1	10.3	14.6
13.0	13.1	12.5	13.1	13.1	8.0	8.2	10.7	12.4	13.8	13.0	16.9	13.1	10.4	10.3	10.0	12.3
11.5	4.8	3.9		10.5	6.6	2.3	9.2	11.0	5.1	10.2	7.1	7.6	8.4	8.2	4.8	7.1
6.5	3.9				6.5		8.0	8.0	2.5	6.6	3.0	6.6	7.6	6.6	3.5	5.8
5.9	3.5				3.2		5.2	7.0		5.9	2.3	4.3	6.6	3.8	2.9	4.8
4.4	2.9						5.2	5.1				2.7	4.1	2.8	2.8	3.8
2.7	2.4						3.9	4.1					2.8	2.3	2.5	3.5
	1.8						2.9	4.0							1.8	2.9
	1.8							2.9							1.7	2.5
								1.1							1.2	1.5
															0.7	0.9

Table II--continued

BstEII + BamHI	BstEII + XhoI	BstEII + HindIII	BstEII + EcoRI	BstEII + HpaJ	BamHI + HindIII	BamHI + EcoRI	BamHI + XhoI	BamHI + HpaI	BamHI + XbaI	BamHI + KpnI	HindIII + XhoI	EcoRI + XhoI	KpnI + XhoI	XhoI + XbaI	HindIII + XbaI	HindIII + EcoRI
19.4	25.5	26.9	24.6	21.7	19.1	19.1	19.1	19.1	19.1	21.1	22.6	22.6	24.3	24.6	42.8	40.4
19.0	22.3	24.3	19.4	16.0	19.0	19.0	16.0	15.7	19.0	19.0	22.6	14.1	14.5	22.3	27.9	17.4
17.7	7.4	11.4	17.4	13.0	16.6	17.4	10.9	13.0	13.0	14.3	15.3	14.0	11.1	14.6	16.9	17.0
11.5	5.4	10.3	12.6	11.0	13.0	13.0	8.7	11.4	11.7	11.1	11.6	12.6	11.0	11.0	8.1	12.7
5.0	5.3	4.8	4.8	9.1	8.7	10.1	8.0	10.7	10.6	6.9	7.9	11.0	10.3	5.6	2.3	11.7
4.8	4.9	4.2	3.4	8.6	8.6	5.9	6.3	9.7	7.3	6.0	5.1	8.0	8.0	5.1	1.3	1.3
3.9	4.1	3.8	2.9	4.8	5.9	5.1	5.7	7.9	6.1	5.6	4.1	5.2	5.2	4.3		
3.5	3.5	3.5	2.9	3.8	4.4	4.4	5.2	6.0	6.0	4.6	3.0	5.1	4.0	4.0		
2.9	3.3	2.9	2.5	3.5	2.8	2.8	5.1	4.4	4.4	4.4	2.9	4.0	3.0	2.9		
2.8	3.3	2.5	1.8	2.9	2.7	2.6	4.2	3.7	2.8	3.1	2.6	2.9	3.0	1.9		
1.8	3.0	1.8	1.7	2.5			4.0	2.9	2.4	2.8	2.3	1.1	3.0	0.8		
1.8	2.9	1.7	1.7	1.8			3.0			2.8			2.5			
1.3	2.5			1.7			3.0						2.3			
1.3	1.8						0.9						1.3			
0.8	1.8															

Data are expressed as "% of genome length." Fragments of less than about 1 map unit would not have been detected.

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2. Wadell, G. and Varsanyi, T.M. (1978) Demonstration of three different subtypes of adenovirus type 7 by DNA restriction site mapping. Infect. and Immun. 21, 238-246.

3. Buescher, M.A., Roninson and Padmanabhan (1981) Physical mapping of human adenovirus type 7 DNA using a simple and sensitive method of terminal labeling. Submitted to Gene.

4. Shinagawa, M. and Padmanabhan, R. (1980) Comparative sequence analysis at the inverted terminal repetitions of different adenoviruses. Proc. Nat. Acad. Sci. USA 77, 3831-3835.

5. Roninson, I. and Padmanabhan, R. (1980) Studies on the nature of the linkage between the terminal protein and the adenovirus DNA. Biochem. Biophys. Res. Commun. 94, 398-405.

6. Tokunaga, O., Shinagawa, M. and Padmanabhan, R. (1981) Physical mapping of the genome and sequence analysis at the inverted terminal repetition of adenovirus type 4 DNA. Manuscript in preparation.

7. Williams, J.F. (1970) Enhancement of adenovirus plaque formation on HeLa cells by magnesium chloride. J. Gen. Virol. 9, 251-255.

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9. Tokunaga, O., Padmanabhan, R., Scott, R.M. and Bancroft, W.H. (1981) A sensitive method for the titration of infectivity of adenovirus types 4 and 7 vaccines. Manuscript in preparation.

Publications Resulted from the Contract Awarded by the USAMRDC

A. Publications

1. Shinagawa, M. and Padmanabhan, R. (1980) Comparative sequence analysis of the inverted terminal repetitions from different adenoviruses. Proc. Nat. Acad. Sci. USA 77, 3831-3835.

2. Roninson, I. and Padmanabhan, R. (1980) Studies on the nature of the linkage between the terminal protein and the adenovirus DNA. Biochem. Biophys. Res. Commun. 94, 398-405.

B. Manuscripts Submitted

1. Buescher, M., Roninson, I. and Padmanabhan, R. (1980) Physical mapping of human adenovirus type 7 DNA using a simple and sensitive method of terminal labeling. Submitted to Gene.

C. Manuscripts in Preparation

1. Tokunaga, O., Padmanabhan, R., Scott, R.M. and Bancroft, W.H. (1981) An improved plaque assay for adenovirus vaccine strains.

2. Tokunaga, O., Shinagawa, M. and Padmanabhan, R. (1980) Physical mapping of the genome and DNA sequence analysis at the inverted terminal repetition of adenovirus type 4.

D. Abstracts of Papers Presented in Meetings

1. Buescher, M.A., Tokunaga, O., Roninson, I. and Padmanabhan, R. (1980) Physical mapping of adenovirus genomes using a simple and sensitive method of terminal labeling. Federation Proceedings Abstracts #1786 meeting held at St. Louis, Mo.).

2. Tokunaga, O., Shinagawa, M. and Padmanabhan, R. (1981) Physical mapping of the genome and sequence analysis at the termini of adenovirus type 4 DNA. 24th West Central States Biochemistry Conference (held at University of Nebraska-Lincoln, on October 23 and 24, 1981) Abstract #

Personnel Supported During July 1, 1979-June 30, 1980

Personnel	Experience	Period supported by contract
Morikazu Shinagawa, D.V.M., Ph.D. Visiting scientist currently Assistant Professor, Obihiro University School of Medicine, Obihiro, Japan.	Virology and Nucleic Acid Structure. Since 1977	July-August, 1979 (End of his two-year stay in this country)
Mrs. Raji Padmanabhan	Tissue culture, Virology and Nucleic Acids Structure. Since 1973	July-September, 1979 (joined NCI)
Mr. Igor Roninson, M.S.	Virology and Nucleic Acids For 5 years	July-September, 1979 (now a graduate student at M.I.T.)
Mr. Charles Burkaw, B.S.	Learning the techniques in Virology.	December-May, 1980
Ms. Monica Buescher, B.S.	First year medical student; three years of experience in Virology & Nucleic Acids at Johns Hopkins University.	January, 1980 Independent research elective study for credits.
Osamu Tokunaga, M.D., Ph.D.	Tissue culture and tumor biology.	January, 1980-present.
Radha Krishnan Padmanabhan, Ph.D. Principal Investigator	Virology and Nucleic Acid Structure and Function	Salary support from Research Career Development Award from National Cancer Institute

Personnel Supported During July 1, 1980-July 31, 1981

Personnel	Experience	Period supported by contract
Osamu Tokunaga, M.D., Ph.D.	Tissue culture and tumor biology	January, 1980-July 31, 1981
Ms. Monica Buescher	Medical Student; three years experience in molecular biology during her undergraduate curriculum at J.H.U., Baltimore, MD.	months of Aug., 1980, and Jan., 1981. She won a Dean's summer fellowship for June and July, 1980.
Ms. Karen Turner	Lab. Assistant (part-time; B.S. degree holder). Learning tissue culture and virology.	Dec. 15, 1980-June 30, 1981
David Weber	Lab. Scientist I (temporary). Finished all his requirements towards M.D. degree.	Jan. 5-Mar. 31, 1981
Sheila Bond, Ph.D.	Tissue Culture and Virology	Jan. 5-Apr. 6, 1981
Morikazu Shinagawa, D.V.M., Ph.D. Visiting Scientist Currently Assistant Professor, Obihiro University School of Medicine, Obihiro, Japan.	Virology and Nucleic Acid Structure Since 1977	March-Apr. 30, 1981
Radha K. Padmanabhan, Ph.D. Principal Investigator University of Maryland School of Medicine	Virology and Nucleic Acid Structure and Function Since 1969	Salary support for two months: Jul. 1-Aug. 30, 1980